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14. ABSTRACT Children and mice with one defective NF1 allele have learning disabilities linked to hippocampal deficits. Compared to wild type mice, expression of the c-met receptor tyrosine kinase is elevated in the hippocampus of mice with astrocyte-targeted disruption of the Nf1 gene. Hippocampal neurons from these mutant mice still express neurofibromin, arguing that increased c-met expression is not due to non-specific targeting of neurons. Both c-met and its ligand, hepatocyte growth factor, promote hippocampal neuron maturation and neuron sprouting. Here, we found that Nf1-null astrocytes do not induce c-met expression in wild type hippocampal neurons in vitro and that neurons from the mutant mice do not maintain elevated c-met expression in culture. However, we did find that mice with constitutively active K-ras mutations, but not mice with H-ras mutations, have elevated hippocampal c-met expression that is similar to that observed in the Nf1 mutant animals. Cell extrinsic factors may therefore influence how loss of neurofibromin in astrocytes regulates c-met expression by hippocampal neurons, and these effects may be dependent on K-ras signaling.					
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Final Progress Report
c-met induction in the hippocampus of NF1-null mice

W81XWH-05-1-0196

January 4, 2006

INTRODUCTION

Children and mice with one defective *NF1* allele have learning disabilities linked to hippocampal deficits (Costa et al., 2002). These learning deficits are a significant problem for some neurofibromatosis 1 (NF1) patients. We had previously found that malignant peripheral nerve sheath tumors from patients with NF1 had constitutively elevated expression of the c-met receptor tyrosine kinase (Su et al., 2004). In an effort to determine if loss of the *NF1* gene product, neurofibromin, was sufficient to induce c-met expression in the nervous system, we examined the brains and spinal cords of mice with astrocyte-targeted *Nf1* mutations (see Bajenaru et al., 2002 for details about these mice). We found that compared to wild type mice, expression of the c-met receptor tyrosine kinase is elevated in the hippocampus of mice with astrocyte-targeted disruption of the *Nf1* gene. Hippocampal neurons from these mutant mice still express neurofibromin, arguing that increased c-met expression is not due to non-specific targeting of neurons. Both c-met and its ligand, hepatocyte growth factor, promote hippocampal neuron maturation and neuron sprouting (Korhonen et al., 2000). Our hypothesis, therefore, was that loss of neurofibromin in astrocytes altered astrocyte-neuron interactions, leading to induced c-met expression in the hippocampus, and that such alterations in c-met expression could contribute to the learning and memory deficits observed in NF1 patients.

BODY

Although we were able to perform the majority of the aims, our findings to date did not support our original hypothesis that astrocytes induce c-met expression in hippocampal neurons through altered expression or secretion of c-met inducing factors. As a result, given the limited funds and time allotted for this concept award, we focused on understanding how c-met expression is induced in hippocampal neurons in vivo. As outlined below, in collaboration with Dr. David Gutmann, we examined different mutant mice with activating mutations in different Ras family members to test if Ras activation was sufficient to induce c-met expression in hippocampal neurons. Our hope is that these studies will serve as the basis for a longer-term study aimed at understanding the consequences of c-met overexpression in the brains of mice with *Nf1* mutations and in patients with NF1.

Objective 1: To test if *Nf1*-null astrocytes directly influence c-met expression by hippocampal neurons in vitro

We found that *Nf1*-null astrocytes do not induce c-met expression in wild type hippocampal neurons in vitro. We cultured *Nf1*-null or wild type astrocytes with either

hippocampal neurons from mice with astrocyte-targeted loss of neurofibromin (*Nf1*^{FL/FL} mice crossed with GFAP-cre mice as previously described; see Bajenaru et al., 2002) or wild type neurons. We repeated these experiments several times, using different ratios of neurons and astrocytes. We also prepared conditioned medium from *Nf1* mutant astrocytes and tested if it could induce c-met expression in hippocampal neurons. Both Western blotting and immunocytochemical assays were used in these studies, utilizing a c-met-specific antibody. We failed to see any induction in c-met expression in any of these experiments (data not shown). Furthermore, we did not observe changes in calbindin D immunoreactivity in these experiments or in situ, using sections from wild type and *Nf1* mutant mice (data not shown). We tested this because previous studies had linked altered c-met activity with alterations in the numbers of calbindin D-immunoreactive cells in the hippocampus.

We did, however, find that mice with constitutively active K-ras mutations, but not mice with H-ras mutations, have elevated hippocampal c-met expression that is similar to that observed in the *Nf1* mutant animals (Fig. 1). Cell extrinsic factors may therefore influence how loss of neurofibromin in astrocytes regulates c-met expression by hippocampal neurons, and these effects may be dependent on K-ras signaling.

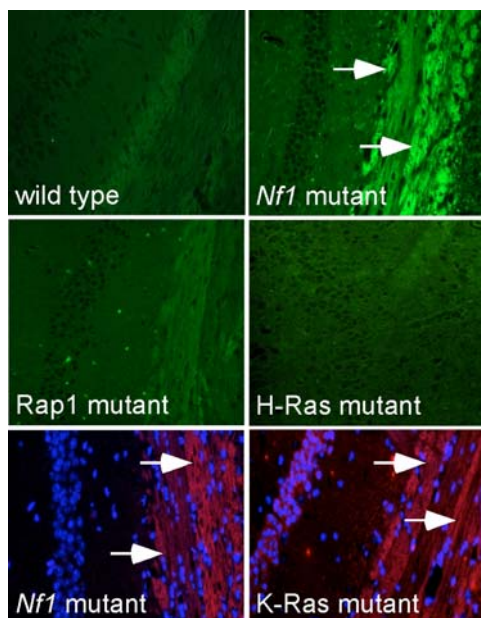


Fig. 1. c-met expression in hippocampal fibers of wild type as compared to different mutant mice. In the **top** and **middle** panels, c-met immunoreactivity (green) is shown in wild type mice, *Nf1* mutant (GFAP-cre x *Nf1*^{FL/FL}) mice, and mice expressing constitutively active Rap1 or H-Ras as indicated. Note that c-met expression is elevated in hippocampal fibers if the *Nf1* mutant mice but not in the other mutant animals as compared to wild type mice of the same age (6 months) and sex (female). In the **bottom** panels, c-met expression (red) is shown in conjunction with DAPI labeling (blue; to identify cell nuclei) in *Nf1* mutant and constitutively active K-Ras mice, as indicated. Note that the elevated c-met expression in K-Ras mice is similar to that observed in *Nf1* mutant mice. All sections include the C3 area of the hippocampus.

Objective 2: To determine whether elevated c-met expression is reversible in hippocampal neurons from mice with astrocyte-targeted *Nf1* mutations and if increased c-met expression is sufficient to induce altered hippocampal neuron phenotypes

Each of the tasks outlined in this objective depended on hippocampal neurons maintaining constitutively elevated c-met expression following dissociation and growth in vitro or on the ability of c-met to be induced in astrocyte-neuron co-cultures. In all of our experiments, we found that neurons from *Nf1* mutant mouse hippocampi expressed the same levels of c-met as wild type neurons in vitro (data not shown). These data suggest that elevated c-met expression by hippocampal neurons in *Nf1* mutant animals may be reversible. Alternatively, the cells that express elevated c-met from these mice

may, for reasons that are unclear, be selected against once that they are placed in culture. Future experiments will be required to distinguish between these possibilities.

KEY RESEARCH ACCOMPLISHMENTS

- Verified that c-met expression is elevated in mice with astrocyte-targeted *Nf1* mutations
- Demonstrated that elevated c-met is not induced in hippocampal neurons by soluble factors synthesized by *Nf1* null astrocytes
- Found that hippocampal neurons in vitro from *Nf1*-mutant mice do not maintain elevated c-met expression, suggesting that the effect may be reversible
- Demonstrated that elevated c-met expression is likely linked to abnormal K-Ras activation in *Nf1* mutant mice

REPORTABLE OUTCOMES

We are continuing these studies and hope to publish a descriptive paper, in collaboration with the laboratory of Dr. David Gutmann at Washington University in St. Louis, in the near future.

CONCLUSIONS

Collectively, our findings to date indicate that loss of neurofibromin in astrocytes somehow induces c-met expression in the hippocampus, likely through a K-Ras-mediated signaling cascade. Future studies should focus on (a) whether c-met is similarly constitutively elevated in hippocampal fibers in patients with NF1; (b) if elevated c-met contributes to learning and memory deficits in *Nf1* mutant mice; and (c) if inhibiting K-Ras activity leads to reduced c-met expression in the hippocampus of *Nf1* mutant mice.

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